USE OF BENZYL ALCOHOL, AND OTHER PHENOLIC PRESERVATIVES TO REDUCE PAIN DURING INTRADERMAL INJECTION

This application claims priority to U.S. provisional application no. 60/457,309, filed March 26, 2003, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to formulations and methods for reducing pain during intradermal injection, in particular the use of benzyl alcohol, phenolic agents and aromatic preservatives for such formulations and methods.

2. Background Information

Anatomically, the outer surface of the body is made up of two major tissue layers, an outer epidermis and an underlying dermis, which together constitute the skin (for review, see *Physiology, Biochemistry, and Molecular Biology of the Skin, Second Edition*, L.A. Goldsmith, Ed., Oxford University Press, New York, 1991). The epidermis is subdivided into five layers or strata of a total thickness of between 75 and 150 μm. Beneath the epidermis lies the dermis, which contains two layers, an outermost portion referred to as the papillary dermis and a deeper layer referred to as the reticular dermis. The papillary dermis contains vast microcirculatory blood and lymphatic plexuses. In contrast, the reticular dermis is relatively acellular and avascular and made up of dense collagenous and elastic connective tissue. Beneath the epidermis and dermis is the subcutaneous tissue, also referred to as the hypodermis, which is composed of connective tissue and fatty tissue. Muscle tissue lies beneath the subcutaneous tissue.

In the past, intradermal delivery of drugs has not been a standard route of administration due to the limiting factors of the intradermal space (i.e. larger volumes in a limited space, difficult technique and discomfort). The few drugs commonly used for intradermal injections have been used for skin infiltration of an anesthetic agent to provide moderate duration anesthesia prior to vascular cannulation. These injections by themselves have proven to be quite painful. In many cases the amount of pain experienced is great enough to cause the recipient to forego the injection altogether. The

addition of various agents to decrease pain upon injection has been limited to these anesthetic agents mainly because of the limited number of compounds delivered via the ID route. But, with the emergence of new and reliable ID delivery devices, and the improved pharmacokinetics, the desire to deliver compounds via this route has increased. Reformulating these compounds to make them "pain free" upon injection is crucial when paired with new intradermal delivery devices.

SUMMARY OF THE INVENTION

In recent clinical trials it has been demonstrated that compounds or solutions containing preservatives in their formulation (bacteriostatic saline, insulin) have significantly lower pain scores for intradermal delivery than preservative-free solutions. The addition of benzyl alcohol (bacteriostatic saline), cresol (insulin) and aromatic preservatives to a formulation of an active compound reduces the subject's perception of pain during intradermal injection, presumably acting as a local anesthetic on the immediate tissue surface within the intradermal space, thereby making the perception of infiltration less noticeable. (It is noted that the operability of the invention is not dependent upon this being the method by which the results are achieved.) As more compounds are being investigated for future ID delivery, re-formulation and patient comfort must be considered. The addition of one of these preservatives to an active compound, which may be uncomfortable upon intradermal delivery, should decrease perception for the patient, enabling more compounds to be delivered via the intradermal route.

Pain upon microneedle/intradermal injection is associated with tissue distention or other physiological or pharmacological affect of the injectate. The addition of a desensitizer, local anesthetic or masking agent to the formulation eliminates these effects for ID administration of drugs. Previous work in this field was directed towards subsequent physical trauma such as cannula placement. The current use of the present agents is directed towards real time masking of drug and other injection effects in the ID space.

Benzyl alcohol, m-cresol and other preservatives were previously thought to be deleterious for inclusion in ID formulations based on their potential for dermal irritancy

measured in animal models such as swine (Draize Irritancy Scores, NB 00-8440-01). In humans, however, these effects are not observed and beneficial results are gained in the perception of reduced pain.

Accordingly, it is an object of the invention to provide formulations and methods for intradermal injection that include a pain-reducing agent selected from the group consisting of pharmaceutically acceptable preservative agents, antimicrobial preservatives, disinfectants, antiseptics, and antioxidants. These compounds have traditionally been included in parenteral formulations for injection for the purpose of protecting, stabilizing, or otherwise preventing degradation of the drug components of an injection during storage or usage. These agents provide a chemical means of preservation by inhibiting microbial growth, and thereby, constraining decomposition of the drug or vaccine product. This preservation process is effective for control of potential pathogens, extension of product shelf life, and protecting against the deleterious consequences of microbial contamination. Antioxidants also provide chemical means of protection of the active drug process, by prevention of oxidative degradation of the active drug process. These pharmaceutical properties may either be invoked separately or in concert by a single agent. Likewise various preservative and antioxidant additives may accomplish these activities by a variety of chemical or biological mechanisms (for reference see: Dermatological Formulations, BW Barry, 1983, Marcell Dekker, New York, NY). While the pharmaceutical properties of these agents are currently beneficial, their usage may not be limited by their current role. Many of the preservative, disinfecting, and antioxidant properties imparted by these agents can alternatively be accomplished by standard physical pharmaceutical processes known to those skilled in the art, such as aseptic or sterile filling and processing, terminal sterilization of products, and/or filling under inert atmosphere or vacuum, thus abrogating the need for their usage. Likewise the usage of preservatives in parenteral formulations for injection has been declining with the advent of single unit dose containers, which are accessed only once for usage, thereby minimizing or eliminating the need for an antimicrobial preservative. It is the object of this invention, to provide formulations and methods, which utilize these pharmaceutically acceptable agents for the alternative purpose of reducing the overall perception of an intradermal injection. It is the purpose of this invention to accomplish this decreased

perception of ID injection using these pharmaceutically acceptable agents, where their usage is not indicated or required for achieving their preservative or antioxidant properties, or where the need for their current usage has been abrogated by other means such as effective pharmaceutical processing methods or unit-dose forms. It is to be particularly noted that modern processing methods and unit dosages have eliminated the need to include such preservatives in many pharmaceutical formulations.

The perception of ID injection (e.g. pain) may be due to physical elements of the injection process or chemical elements of the drug formulation including the active drug component or other excipients present within the drug vehicle. Physical elements of the ID injection perception process which may be masked by inclusion of an appropriate pharmaceutical additive include but are not limited to device application to the skin, insertion of needles, microneedles, microcannula or other dermal accessing means, the perception of tissue distension or increased dermal pressure upon injection, or mechanical tissue damage at the injection site caused by the injection process. Chemical and biochemical elements of the ID injection perception process which may be masked by inclusion of an appropriate pharmaceutical additive include but are not limited to: disruption of local ion channel flux, variations in local tonicity or pH at the injection site, stimulation of local or systemic immunological processes such as mast cell degranulation, stimulation of dermal nociceptors, localized vasoconstriction or vasodilatation, or localized tissue damage or necrosis due to the chemical effects.

Particularly preferred as agents are those selected from the group consisting of alcoholic preservatives (e.g. ethanol, isopropropanol, chlorbutanol, benzyl alcohol, phenoxyethanol, phenylethyl alcohol, bronopol, monothioglycerol, propylen glycol, xylitol, glycerol), acidic preservatives including inorganic and organic salts thereof (benzoic acid, lactic acid, propionic acid, sorbic acid, also sodium or potassium salts thereof), phenolic and paraben preservatives (phenol, chloroxylenol, m-cresol, o-cresol, p-cresol, chlorocresol, thymol, anisol, butylated hydroxyanisol, propyl gallate, methyl paraben, ethyl paraben, propyl paraben, butyl paraben, and halogenate derivatives thereof), quaternary ammonium preservatives (benzalkonium chloride), other preservatives (chlorhexidine, vanillin), phenolic antioxidants (butylated hydroxyanisole, butylated hydroxytoluene, nordihydroguiaretic acid, propyl gallate, pyrogallol) organic

acid, alcohol, and ester antioxidants (ascorbic acid, citric acid, malic acid, sorbitol, glycerol, propylene glycol, ascorbyl palmitate) and quinone antioxidants (alpha tocopherol, hydroquinone, hydroxycoumarins)..

Particularly preferred embodiments of the invention include current compounds approved for parenteral administration by the FDA or other regulatory body. It is anticipated that compounds containing benzyl, phenol, aromatic, or polyaromatic ring structures may exhibit these anesthetic and analgesic effects for ID administration. Preferred benzylic compounds (containing a benzyl structure) include benzylic alcohols and benzylic acids. Preferred phenolic compounds (containing a phenol structure) include phenolic alcohols, phenolic acids and paraben.

The concentration of such agents in the formulation is generally anticipated to be less than about 10% (w/v), preferably less than 5%, more preferably less than or equal to 2%, most preferably less than or equal to 1% (typically 0.25% or less). Useful working ranges and optimal amounts can be determined by persons of skill in the art without undue experimentation.

The above-mentioned pain-reducing agents can be included in any formulation that is suitable for, and desired to be used for intradermal injection, including diagnostic, prognostic, prophylactic and therapeutic formulations. These include diagnostic agents, drugs, and other substances which provide therapeutic or health benefits such as for example nutriceuticals. Diagnostic substances useful with the present invention include antibodies or antigens, macromolecular substances such as, for example, insulin, ACTH (e.g. corticotropin injection), luteinizing hormone-releasing hormone (e.g., Gonadorelin Hydrochloride), growth hormone-releasing hormone (e.g. Sermorelin Acetate), cholecystokinin (Sincalide), parathyroid hormone and fragments thereof (e.g. Teriparatide Acetate), thyroid releasing hormone and analogs thereof (e.g. protirelin), secretin and the like. Agents may be labeled (e.g. with radioisotopes or fluorescent tags) or unlabeled.

Therapeutic substances which can be used with the present invention include Alpha-1 anti-trypsin, Anti-Angiogenesis agents, Antisense, butorphanol, Calcitonin and analogs, Ceredase, COX-II inhibitors, dermatological agents, dihydroergotamine, Dopamine agonists and antagonists, Enkephalins and other opioid peptides, Epidermal

growth factors, Erythropoietin and analogs, Follicle stimulating hormone, G-CSF, Glucagon, GM-CSF, granisetron, Growth hormone and analogs (including growth hormone releasing hormone), Growth hormone antagonists, Hirudin and Hirudin analogs such as Hirulog, IgE suppressors, Insulin, insulinotropin and analogs, Insulin-like growth factors, Interferons, Interleukins, Luteinizing hormone, Luteinizing hormone releasing hormone and analogs, Heparins, Low molecular weight heparins and other natural, modified, or synthetic glycoaminoglycans, M-CSF, metoclopramide, Midazolam, Monoclonal antibodies, Peglyated antibodies, PEGylated proteins or any proteins modified with hydrophilic or hydrophobic polymers or additional functional groups, Fusion proteins, Single chain antibody fragments or the same with any combination of attached proteins, macromolecules, or additional functional groups thereof, Narcotic analgesics, nicotine, Non-steroid anti-inflammatory agents, Oligosaccharides, ondansetron, Parathyroid hormone and analogs, Parathyroid hormone antagonists, Prostaglandin antagonists, Prostaglandins, Recombinant soluble receptors, scopolamine, Serotonin agonists and antagonists, Sildenafil, Terbutaline, Thrombolytics, Tissue plasminogen activators, TNF - , and TNF - antagonist, the vaccines, with or without carriers/adjuvants, including prophylactics and therapeutic antigens (including but not limited to subunit protein, peptide and polysaccharide, polysaccharide conjugates, toxoids, genetic based vaccines, live attenuated, reassortant, inactivated, whole cells, viral and bacterial vectors) in connection with, addiction, arthritis, cholera, cocaine addiction, diphtheria, tetanus, HIB, Lyme disease, meningococcus, measles, mumps, rubella, varicella, yellow fever, Respiratory syncytial virus, tick borne Japanese encephalitis, pneumococcus, streptococcus, typhoid, influenza, hepatitis, including hepatitis A, B, C and E, otitis media, rabies, polio, HIV, parainfluenza, rotavirus, Epstein Barr Virus, CMV, chlamydia, non-typeable haemophilus, moraxella catarrhalis, human papilloma virus, tuberculosis including BCG, gonorrhoea, asthma, atheroschlerosis malaria, E-coli, Alzheimer's Disease, H. Pylori, salmonella, diabetes, cancer, herpes simplex, human papilloma and the like other substances including all of the major therapeutics such as agents for the common cold, Anti-addiction, anti-allergy, anti-emetics, anti-obesity, antiosteoporeteic, anti-infectives, analgesics, anesthetics, anorexics, antiarthritics, antiasthmatic agents, anticonvulsants, anti-depressants, antidiabetic agents,

antihistamines, anti-inflammatory agents, antimigraine preparations, antimotion sickness preparations, antinauseants, antineoplastics, antiparkinsonism drugs, antipruritics, antipsychotics, antipyretics, anticholinergics, benzodiazepine antagonists, vasodilators, including general, coronary, peripheral and cerebral, bone stimulating agents, central nervous system stimulants, hormones, hypnotics, immunosuppressives, muscle relaxants, parasympatholytics, parasympathomimetrics, prostaglandins, proteins, peptides, polypeptides and other macromolecules, psychostimulants, sedatives, and sexual hypofunction and tranquilizers.

Particularly preferred for use in the invention are fertility compounds (such as Antagon™, Bravelle™, Centrotide®, Follistim®, Gonal-F®, Fertinex®, Novarel™, Ovidrel® Pregnyl®, and Repronex®), antiemetics (e.g. Compazine®, Tigan®, Kytril®, Zofran®),vasoconstrictors, vasodilators, emulsions or other compositions containing particles, compositions of high viscosity, PEGylated compounds, gels, morphine and other opiod pain relievers, triptans (e.g. sumatriptan), local anesthetics (such as lidocaine), injectable sildanafil and similarly acting compounds.

It will be clear that the invention is especially useful for ID injections that have a high degree of pain, irritation or discomfort. Thus, the invention will be especially useful for ID bolus injections wherein greater than 100 ul, preferably 200ul, more preferably 250 ul, and most preferably 500 ul is delivered per needle, and for substances that are typically irritating (e.g. heparin, low molecular weight heparin, triptan antimigrane compounds, and COX-2 inhibitors).

As used herein, "intradermal delivery" (ID) means the delivery of materials to the intradermal space and its interrogation as described by Pettis *et al.* in WO 02/02179 A1 (PCT/US01/20782 having a priority date of June 29, 2000). "Bolus" delivery is defined as occurring over a period of less than 10 minutes.

Micro-cannula- and microneedle-based methodology and devices are described in U.S. Application Serial No. 606,909, filed June 29, 2000. Standard steel cannula can also be used for intra-dermal delivery using devices and methods as described in U.S. Serial No. 417,671, filed October 14, 1999. These methods and devices include the delivery of substances through narrow gauge (30G or narrower) "micro-cannula" with a limited depth of penetration (typically ranging from 10 μm to 2 mm), as defined by the total

length of the cannula or the total length of the cannula that is exposed beyond a depth-limiting hub feature.

Advantages and improvements of the present invention over currently used formulations and methods include reduced pain, enhanced delivery, and improved compliance. The additive suppresses the perception of the delivery process in a very transient and rapidly resolving manner, avoiding extended anesthesia such as that achieved with Lidocaine or other local anesthetics, which is to be avoided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the distribution of individual scores from a representative intradermal injection of preservative-free saline, as described in Example 3.

Figure 2 shows the distribution of individual scores from a representative intradermal injection of saline containing the preservative benzyl alcohol as described in Example 4.

Figures 3A and 3B show calculated 95% confidence intervals for pain at intradermal device insertion (needlestick) and upon completion of saline containing the preservative benzyl alcohol as described in Example 4.

DETAILED DESCRIPTION OF THE INVENTION

METHODS

A series of prospective, randomized, open label human clinical studies were conducted. The following inclusion criteria was typically used:

18 years of age or older
In stable health status with no acute or severe illness
Willing to complete all study procedures
Available for designated study days

Persons with certain criteria (e.g. pregnancy, lactation, antibiotic therapy, etc.) were typically excluded from the study.

Typically, each participating subject received a series of 'treatments' consisting of intradermal infusions or injections of either sterile unpreserved isotonic saline, or sterile bacteriostatic isotonic saline for injection in the anterior thigh using microneedle-based intradermal injection devices. Participants were asked to rate the pain immediately after the device was applied to the skin, and at the end of the injection/infusion procedure. In

addition, injection sites were evaluated for irritation using the Draize dermal irritation scoring scale for erythema and edema, and for the presence of any other adverse skin effects, occurring at the time of injection or developing at a later time.

Intradermal injection devices were varied in design, both for the number of microneedles inserted into the dermis and the usable length of the microneedles for a given device. Typical intradermal injection devices consisted of either 1 or 3 microcannula, with a 1-1.5 mm usable length. For example, the notation 1 X 1mm indicates 1 microneedle with a 1mm usable length. The injection sight for intradermal administration was the upper aspect of either the left or right thigh, and sites were randomized according to a predetermined dosing schedule. During intradermal administration the rate and volume of saline delivery were controlled by means of a highly accurate syringe pump, flowing at a programmed constant rate.

The number of study subjects varied for different studies, as did the male/female distribution ratio. Study subjects consisted of compensated healthy male and female volunteers who were admitted based on the above representative inclusion/exclusion criteria, signed an informed consent for participation, and completed all aspects of the trial.

The examples below are from separate independent clinical studies, but are representative of outcomes routinely obtained using the device characteristics and injection methods stated. Likewise the cited examples, while not identical in method (e.g. total infusion volume) serve to illustrate the beneficial differences and altered perception outcomes encountered when an anesthetic or analgesic compound of the invention is incorporated into an intradermally administered formulation.

Pain Scores for all Studies

Pain was determined using a Gracely Box SL Scale (reference: Heft, Gracely and Dubner, *Pain*, 1980, 9:363-373). Pain scores were typically recorded after insertion of the intradermal delivery device, and upon complete intradermal instillation of the total saline dose. The Gracely Pain Scale is a 21 point scoring scale (0-20) with levels that go from 0

or *No Pain sensation*, up to 20, two levels above *extremely intense.*

Table I-Gracely Pain Scale

20	
19	
18	Extremely intense
17	Very intense
16	Intense
15	Strong
14	
13	Slightly intense
12	Barely strong
11	Moderate
10	
9	
8	Mild
7	Very mild
6	
5	Weak
4	Very weak
3	
2	
1	Faint
0	No pain sensation

Typically, pain scale scores were analyzed by ANOVA using a linear-type model. Post-hoc multiple comparisons were performed if the factor effects or interaction were significant. The post-hoc comparisons helped to identify which treatments actually differ from each other and by how much on average (with 95% confidence interval). The assumptions underlying these computations were that the noise was normally distributed and that the variance was constant for all experimental conditions

Examples 1-3

These examples were from three independently performed clinical trials using the delivery conditions stated in Table 2. Intradermal administration of various volumes of non-preserved saline was performed into the thighs of from 12-20 subjects using the indicated intradermal device and delivery conditions. The saline used in these studies did not contain any of the additives responsible for reducing the perception of the intradermal delivery process. The mean and median scores for pain perception rated on the Gracely

pain scale are reported in Table 3. Scores are reported for both the insertion of the intradermal delivery microcannula alone prior to administration of any saline, and for end of injection pain perception, which rates the overall pain of the intradermal saline delivery process.

These examples illustrate that the insertion of a microneedle based delivery device typically ranks very low on the pain perception scale. Most mean and median device insertion scores ranked between 0-2 on the Gracely perception scale, near the "faint" reporting region. Upon complete administration of the saline dosage all studies showed a statistically significant increase in reported perception scores. Mean scores ranked between 4-9 for the intradermal administration process, corresponding to the "weak" to "mild" range of the Gracely scale. These independently gathered data indicate that although the device systems may be minimally perceptible, the administration process is responsible for the increase of perceived pain, even for low administration volumes.

In addition a dotplot showing the distribution of end of injection scores from all subjects in Example 3, Conditions E and F is shown in Figure #1. Statistical ANOVA analysis of the response data, indicate a statistically significant subject-to-subject difference for end of injection pain perception. This is reflected in the wide distribution of perception scores seen in Figure 1. This subject-based difference was also statistically significant for the conditions tested in Examples 1 and 2, with a similar wide distribution for end-of-injection scoring (data not shown).

Table 2.

	Device Configuration	Saline Volume (μL)	Saline Delivery Rate (μL/min)	Saline Delivery Duration (min)	# Study Subjects
Example 1					
A	1X1mm	250	50	5	12
B	1X1mm	300	50	5.5	12
Example 2					
C	3X1mm	100	50	2	16
D	3X1mm	100	100	1	16
Example 3					
E	1X1mm	100	100	1	20
F	3X1mm	100	100	1	20

Table 3.

	Intradermal Device Insertion Pain		End of Injection Pain	
Condition	Mean	Median	Mean	Median
Example 1				
A	1.0	0	6.7	8
B	1.4	0	4.8	5
Example 2				
C	3.7	3	7.3	7.5
D	1.9	0	8.4	10
Example 3				
E	1.2	1	7.3	7
F	2.2	1	7.9	8

Example 4

In an independently performed study in 20 subjects, intradermal injections were made using sterile saline for injection containing 0.9% benzyl alcohol, under the conditions of Table 4. The study was performed using methods and techniques as reported above and

similar to those used in Examples 1-3, with the principle variation being the use of a pain-reducing additive in the administered solution.

Table 4.

	Device Configuration	Saline Volume (μL)	Saline Delivery Rate (μL/min)	Saline Delivery Duration (min)	# Study Subjects
Example 4					
G	3 x 1.0mm	600	100	6	20
H	3 x 1.5mm	600	100	6	20
I	3x 1.0mm	400	100	4	20
J	3 x 1.5mm	400	100	4	20

Table 5.

	Intradermal Device Insertion Pain		End of Injection Pain	
Condition	Mean	Median	Mean	Median
Example 4				
G	1.8	1.0	1.4	0
H	2.2	1	0.8	0
I	1.8	1	0.7	0
J	1.4	0	1	0

It can be seen that the pain scores after infusion process for 1mm and 1.5mm needle length intradermal delivery devices were much lower in this Example compared to the prior three examples, despite an increase in administered volume. In fact mean pain scores after administration ranked near the "faint" range of the Gracely perception scale, while median scores ranked as "no pain perception".

A dotplot showing the distribution of individual pain scores at end of injection for conditions G-J of Example 4 is shown in Figure 2. Statistical ANOVA analysis of the perception score was performed on the data from Example 4. The ANOVA model

included subject-to-subject differences, nurse effect, time recorded (needle stick or after entire dose), order of injection, leg (R or L), needle lengths, volume and the needle lengths by volume interaction. The ANOVA results showed that the average pain after the needle stick was significantly higher by 0.8 pain scale units (with 95% CI of (0.3, 1.4)) than the average pain after the entire dose. The only other detected significant effect was the subject effect at either needle placement or end-of-dose. Overall, the subject variability was also decreased as shown by the tight clustering of reported scores seen in Figure 2.

Delivery of high dose volume to the ID space in the human clinical trials of Example 4 resulted in an exceptionally low perception of pain when including pain reducing agents of the invention. Scores were substantially lower than all previous delivery to this tissue space and even below that of microneedle insertion. All scores ranked as imperceptible or nearly imperceptible on a 21-point Gracely Pain Scale.

A comparison of average pain per injection type is shown in Figures 3A and 3B Figures 3A and 3B show individual 95% confidence intervals for needle stick pain and pain after the administration of the entire dose per device. It should be noted that in the previous examples 1-3, participant's rated the pain after the entire dose had been delivered as significantly higher than the pain after the initial needle stick.

Each of the aforementioned examples employed injectates that were "pharmaceutically accepted vehicles without biologically active medicaments", e.g., isotonic saline. The injectate used in Example 4 was Abbott Laboratories Bacteriostatic 0.9% sodium chloride, injection, USP. The injectate of Example 4 contained 9 mg/ml of benzyl alcohol in addition to saline. Examples 1 through 3 used an injectate which was a preservative-free saline that contains no benzyl alcohol.

The embodiments illustrated and discussed in the present specification are intended only to teach those skilled in the art the best way known to the inventors to make and use the invention, and should not be considered as limiting the scope of the present invention. The exemplified embodiments of the invention may be modified or varied, and elements added or omitted, without departing from the invention, as appreciated by those skilled in the art in light of the above teachings. It is therefore to be understood that, within the scope of the claims and their equivalents, the invention may

be practiced otherwise than as specifically described. Patents and references cited herein are hereby incorporated by reference.